REMARKS

Rejection under §101

It is alleged in the Office action that the "claimed library of individual proteins ... would read on a naturally occurring mixtures of proteins naturally present in e.g., mammalian body." See, Office action dated November 21, 2005, Page 4, lines 1-12. However, the examiner has clearly misconstrued the claims. "Claim terms 'are examined through the viewing glass of a person skilled in the art.'" *Ferguson Beauregard/Logic Controls v. Mega Sys., LLC*, 350 F.3d 1327, 1338 (Fed. Cir. 2003). "Importantly, the person of ordinary skill in the art is deemed to read the claim term not only in the context of the particular claim in which the disputed term appears, but in the context of the entire patent, including the specification." *Phillips v. AWH*, 415 F.3d 1303; 75 U.S.P.Q.2d 1321 (Fed. Cir. 2005). The specification clearly indicates that the claimed library is not comprised solely of naturally-occurring proteins as alleged in the Office action, but is comprised of different individual elements to form protein structures which are not found in nature. The methods described in the specification for creating libraries involve the so-called "hand-of man," e.g., utilizing genetic engineering steps. See, *Diamond, Commissioner of Patents and Trademarks v. Chakrabarty*, 447 U.S. 303, 206 USPQ 193 (1980). Although unnecessary, the claims have been amended to indicate that the claimed polypeptides are recombinant. The specification is replete with references to the term "recombinant" to point out that the claimed library is formed by combining different protein elements to form novel

structures. See, e.g., Specification, Page 1, line 22; Page 2, line 26; Page 10, line 27; Page 11, line 12; Page 30, line 38.

Rejection under §112, first paragraph

The examiner apparently has overlooked the fact that Applicant is claiming a general method of identifying individual proteins which bind to a target of interest. Claims 64-74 are directed to these methods, and claims 52-63 are directed to the libraries utilized in the methods. The examiner's application and understanding of the written description guidelines is completely out of line, and at odd with, the policy purposes for which they were written. *The Regents of The University of California v. Eli Lilly*, 119 F.3d 1559; 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997) (the "*Lilly* case"), and the related cases that invoked the written description requirement in invalidating patent claims, involved subject matter where the novelty resided in the complete sequence of the individual DNAs that were claimed. According to the Federal Circuit, the specification in the *Lilly* case described only a single species (rat insulin cDNA) that fell within the scope of the claim, and that was not an adequate description for the generic invention (vertebrate or mammalian insulin cDNA) which was being claimed. The defect in the specification, according to the *Lilly* court, was that

> In claims to genetic material, however, a generic statement such as 'vertebrate insulin cDNA' or 'mammalian insulin cDNA,' without more, is not an adequate written

description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others.

The pending claimed subject matter does not involve the same principles in the *Lilly* case. The claimed elements are not defined only by function. Their structural features are either described in the specification (e.g., "identifier sequence amino acid tracts") or were available to the skilled worker at the time the application was filed (e.g., "protease sensitive sites" and "individual proteins ... being able to bind to a target of interest"). The latter two categories are well known in the art, providing the skilled worker with an instant vision of numerous species and their structures that fall within the claim scope and which distinguish them structurally from others. The identifier tracts are essentially arbitrary sequences of amino acids. Together, the skilled worker can easily envision a legion of species that fall within the claims. Unlike the *Lilly* case where very specific narrow structures were necessary to meet the claim requirements, the present invention involves sequences that were either known in the art or which have only minimal requirements to satisfy the claims.

"Identifier sequence amino acid tracts"

As explained in the specification and summarized in the Brief on Appeal, the identifier

sequence tracts are used to mark and identify the presence of the polypeptide to which they are attached. Protease treatment releases an identifier tract, facilitating the identification of the protein to which it is attached. These amino acid sequences are arbitrary, and there is no required structure recited in the claims as longas they are "unique."

The reference to the *Lilly* case is improper for several reasons. In *Lilly*, the claims were directed to mammalian insulin. Only a miniscule fraction of possible sequences could satisfy the claim, i.e., to encode a polypeptide sequence which possessed insulin activity. Thus, the question was whether the specification provided enough of a description to pick out those sequences with the insulin activity and distinguish them from other sequences. Without a specific generic structure, the court held the claims lacked written description.

The claimed invention involves a different issue. An identifier tract is arbitrary, and can possess substantially any amino acid sequence – including random or semi-random sequences. See, e.g., Specification, Page 2, lines 36-Page 3, line 5. It is not necessary for the tract have a biological activity in the sense of an insulin molecule. To the contrary, it is the sequence itself which has the function as a marker. Thus, any sequence of, e.g., eight amino acids, when uniquely adjacent to a protein of interest, can be used to mark that protein.

How to make and use the identifier sequence amino acid tracts (also known as "barcodes") is fully described in the specification. The barcodes are encoded for by oligonucleotides that are attached, in open reading frame, to the nucleotide sequence encoding

the polypeptide of interest. A protease sensitive-site is placed between them to facilitate release of the peptide identifier tract. The design of the identifiers, including specific structures, is enabled thoroughly in the specification. For example, on Page 4, lines 23-44, an eight-amino acid barcode sequence using 17 of the 20 natural amino acids is described. A specific example is provided of a family of peptide barcodes and their corresponding oligonucleotide sequences. See, Page 5, lines 1-35. Example 2, beginning on Page 29 of the specification, provides a specific working example of barcode sequence. See, Page 30, lines 5-15. Another example is disclosed on Page 35, lines 30-35. Thus, the enablements requirements are clearly met, as well.

"Individual proteins ... being able to bind to a target of interest"

The examiner states that the specification does not provide enough guidance or description for the structure of the specific "individual proteins" which are recited in the claims. On Page 7 of the Office action, it is stated:

> The issue is whether this single protein is representative of the huge scope of the claimed protein library comprising different types of barcodes of no define sequences. It is not apparent how the function of the single protein species, ScFv can be correlated to the huge scope of a protein such as growth hormone, viral, bacterial, tumor and etc. since these proteins differ in structure with ScFv.

This reasoning is flawed. The structural characteristics needed to confer binding activity are well known in the art for binding molecules. It is not necessary to recite these specific structural characteristics where a technology is mature and well developed. Functional language is sufficient. This was expressly stated in *Enzo Biochem Inc.* v. *Gen-Probe*, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002). In discussing the Patent Office's standards for determining compliance with §112, first paragraph, the court stated:

> For example, the PTO would find compliance with §112, P 1, for a claim to an "isolated antibody capable of binding to antigen X," notwithstanding the functional definition of the antibody, in light of "the well defined structural characteristics for the five classes of antibody, the functional characteristics of antibody binding, and the fact that the antibody technology is well developed and mature." Synopsis of Application of Written Description Guidelines, at 60, available at http://www.uspto.gov/web/patents/guides.htm ("Application of Guidelines"). Thus, under the Guidelines, the written description requirement would be met for all of the claims of the '659 patent if the functional characteristic of preferential binding to N. gonorrhoeae over N. meningitidis were coupled with a disclosed correlation between that function and a structure that is sufficiently known or disclosed. We are persuaded by the Guidelines on this point and adopt the PTO's applicable standard for determining compliance with the written description requirement.

"Protease sensitive sites"

The bottom line is that protease sensitive sites are well known in the art, and readily could be envisioned by the skilled worker. Possession of this aspect of the claimed invention was clearly in the inventors' hands on the application filing date. This position was argued in the Brief on Appeal and is hereby incorporated by reference.

The examiner's response set forth on Pages 8-10 of the Office lacks credibility and scientific basis. Many protease sensitive sites are known in the prior art. Such sites are used conventionally in molecular biology, e.g., to cleave tag sequences (that are used in protein purification) from proteins of interest. For example, there are numerous protease enzymes and their protease sensitive cleavage sites in commercial use. For example:

- Promega (1994) (Exhibit 2) describes the uses of a Factor Xa cleavage site
- Pierce (Exhibit 3) describes the use of Factor Xa, Staphylococcus Protease, Submaxillaris Protease, and TPCK trypsin as having specific cleavage sites that can be incorporated into recombinant proteins in order to remove fusion tags
- New England Biolabs (Exhibit 4) similar shows the use of Factor Xa, enterokinase, and genease cleavage sites in protein fusions

These exhibits clearly establish the commercial use and general acceptance of protease sensitive sites to provide a precise cleavage position to cleave off a leader amino acid sequence from a recombinant protein. Indeed, the examiner alleges that cited prior art references (e.g., Knappik, Ring, and Markland) establish that protease cleavage agents were utilized successfully prior to the filing date. See, e.g., Office action, Pages 21-23. The examiner's attempt to imbue ambiguity and unpredictability into this concept is clearly at odds with the state of the art, including her own characterization of it. The examiner's "analysis" of the Eaton reference – allegedly for teaching the impracticability of using Factor Xa as a protease – is irrelevant in view of the fact that this enzyme (and its corresponding cleavage site), as shown in Exhibits 2-4, is utilized commercially in protein purification. Thus, the alleged unpredictability and discrepancies described by the examiner are misleading and a red herring. In reality and in practice, Factor Xa sensitive sites are commonly used in protein purification.

The *Capon* case

A Supplemental Appeal Brief was previously filed to bring the recent decision in *Capon v. Eshar*, 76 USPQ2d 1078 (Fed. Cir. 2005) to the attention of the examiner. The examiner dismissed this case, arguing that the facts were not similar to those at issue here. However, the examiner failed to specifically point out the alleged differences.

Attached are Pages 1080-81 of the *Capon* case. The Eshhar claims, for example, are not

restricted to only antibodies, but also have additional recited elements, including a second gene encoding two distinct domains ("transmembrane" and "cytoplasmic") and an optional third domain ("extracellular"). None of the four possible protein elements (antibody, transmembrane, cytoplasmic, and extracellular) present in Eshhar's chimeric gene were associated with specifically recited structural features. The Federal Circuit did not find this lack of structural information in the claim or specification to make the claim invalid, but rather concluded that precedent did not establish a *per se* rule require the recitation in the specification of sequences that were known in the art prior to the application filing date. Moreover, the court recognized the claims are being generic, and set forth (at 1086) the proper standard for compliance:

> Precedent illustrates that the determination of what is needed to support generic claims to biological subject matter depends on a variety of factors, such as the existing knowledge in the particular field, the extent and content of the prior art, the maturity of the science or technology, the predictability of the aspect at issue, and other considerations appropriate to the subject matter.
>
> ...
>
> It is not necessary that every permutation within a generally operable invention be effective in order for an inventor to obtain a generic claim, provided that the effect is sufficiently demonstrated to characterize a generic invention. See In re Angstadt, 537 F.2d 498, 504 (CCPA 1976).

The facts at issue in this pending application parallel those of *Capon* where it is not the specific individual elements which are being claimed as necessarily novel, but it is the claimed chimeric structure and how these individual elements are arranged which provides novelty and unobviousness. Protease sequences, binding proteins, and arbitrary sequences are known, the technology of recombining sequences is mature, and the predictability factor is high. There is no basis to sustain this rejection. In essence, the present examiner's entire premise is inconsistent with federal law.

Enablement aspects

Given the mature state of the art and the availability of the recited elements in the claims, it is a puzzle what the examiner finds lacking. As indicated previously and above: 1) methods of producing identifier tracts are fully enabled in the specification; 2) the use of protease sensitive sites in fusion proteins is known and predictable; 3) binding proteins are also known, including their sequences. The examiner has failed to find any specific fault with the patent application disclosure.

Rejections under §102

A prima facie case of anticipation was not established in the §102 rejections set forth on Pages 14-18 of the Office action. The examiner failed to identify how the proteins described in

the cited publications anticipate the claimed invention. The examiner has merely given a description of allegedly what is described in the reference, without specifically pointing out how such disclosure allegedly meets all elements of the claimed invention. It is the examiner's burden to make a prima facie case. Since such case has not been made, the rejections must be withdrawn.

Chen et al.

The library described by Chen et al. differs from what is claimed. As described in the Experimental Procedures and illustrated in Fig. 1 of Chen et al., the tethered ligand described by the authors comprises, the following elements in the recited order: CD8 (transmembrane)-**ligand**-thrombin domain cleavage site-ATE (amino terminal domain). Cleavage at the thrombin domain results in the release of a peptide sequence from the ATE domain. This ATE peptide has no identifier purposes, and it is the same for all ligands. Thus, the proteins described by Chen et al., do not provide "individual identifier sequence amino acid tracts which are unique to said individual protein."

Mathews and Wells

Mathews and Wells (Science, 260: 1113, 1993) describe a method for identifying protease substrates using a phage display method. The method involved the phage display of

proteins which contained putative protease cleavage sites. The phage were immobilized through affinity binding of a phage surface human growth hormone (hGH) domain that was fused in-frame to the putative cleavage site. After binding of the hGH domain to immobilized hGH binding protein, the complex was contacted with a protease of interest under conditions effective for the protease to cleave at the cleavage site. When the putative site was sensitive to the protease activity, the phage was released into the medium and collected. See, Abstract; Page 1114, Column 1; Table 1. Cleaved phage were then directly sequenced to determine the amino acid sequence of the cleavage site.

The protein construct utilized by Mathews and Wells containing the following elements: hGH domain-protease substrate-M13 phage protein. See, Mathews and Wells, Fig. 1. There is "no individual identifier sequence amino acid tracts which are unique to said individual proteins." After cleavage at the protease sensitive site ("substrate"), the phage were collected and the site was subsequently determined directly by sequencing, not by the presence of identifier tracts. Moreover, the construct disclosed by Mathews and Wells does not contain unique identifier tracks which, "when bound to the specific target of interest [i.e., hGH binding protein]," "are flanked by one or more protease sensitive sites." Compare pending Claim 52. To the contrary, when the protein described by Mathews and Wells is bound to the hGH binding domain, there is no unique sequence in it which is also "flanked" by a protease sensitive site. The hGH and the M13 domains are present in each and every phage display protein utilized in

the system, and are not unique to the protease substrate.

Georgiou et al.

U.S. Pat. No. 5,866,344 (hereinafter "the '344 Patent") describe a method of displaying polypeptides on the surface of bacterial cells. See, e.g., '344 Patent, Column 3, line 65-Column 4, line 57; Column 10, lines 44-45. Example 3 (Column 27), relied upon in the Office action, incorporates a cleavage site into the displayed antibody in order to facilitate its release from the cell surface. The fusion protein disclosed in the '344 Patent in Example 3 has the following structure: Lpp/Omp-cleavage site-scF (single chain antibody. There are no individual identifier sequence amino acid tracts which are unique to said individual proteins (e.g., "scF"). Moreover, there is no unique sequence in the fusion protein which is also "flanked" by a protease sensitive site. Identifier amino acid tracts are not used to identify the antibody. To the contrary, the antigen – when bound to the antibody on the surface of the cell – is used to identify the clones of interest. See, e.g., Column 6, lines 4-10. Cleavage is only used for purification purposes, not to release an identifier sequence tract.

Rejection under §103

The Knappik patent describes a library of modular DNA sequences that code for antibodies (and other proteins), which contain nucleotide cleavage sites, not protease sensitive

site, that separate sub-sequences in the DNA. These sub-sequences correspond to different protein coding domains. See, Knappik, e.g., Column 8, lines 20-41. The DNA cleavage sites are utilized to selectively modify domains within the coding DNA sequence. See, e.g., Knappik, e.g., Column 10, line 656-Column 11, line 7. See, also, e.g., Knappik, Column 11, lines 40-50; Column 12, lines 22-42. It is unclear why a four-page discussion (Pages 18-21) of this particular disclosure has been included in the Office action, since the examiner never explains its relevance to the claimed invention.

At Column 11, lines 9-40, Knappik suggest additional moieties, including FLAG tags. The FLAG tag arrangement, as explained in Hopp et al., includes a "marker" segment (FLAG tag) and a protease cleavage site, e.g., enterokinase. See, e.g., Hopp et al., Page 1205. The marker sequence can be used for purification purposes, e.g., by providing a binding site for an antibody. However, a FLAG tag and an accompanying cleavage site, does not disclose or suggest the claimed arrangement, e.g., "proteins comprising ... one or more individual identifier sequence amino acid tracts which are unique to said individual protein when bound to the specific target of interest, and are flanked by one or more protease sensitive sites." Compare pending Claim 52. The FLAG tag is not described as unique to the individual proteins, but is the same for each one.

No motivation with an expectation of success has been provide in the rejection for modifying Knappik et al. to have arrived at the present invention. Ring et al. (described on Pages

21-22 of the Office action) and Markland are cited for their disclose of cleavage sites, including cleavage sites adjacent to sFV polypeptides. Hutchens (U.S. Pat. No. 6,734,022) is cited for its MALDI-TOF disclosure. None of the cited references describe one or more individual identifier sequence amino acid tracts which are unique to said individual protein when bound to the specific target of interest. Compare Claim 52. Moreover, the examiner makes no attempt to supply this feature from the prior art. The examiner has clearly failed to set forth a prima facie case of obviousness.

In view of the above remarks, favorable reconsideration is courteously requested. If there are any remaining issues which could be expedited by a telephone conference, the Examiner is courteously invited to telephone counsel at the number indicated below.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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EXHIBIT

2

Promega Notes Magazine Number 48, 1994, p.26

Technically Speaking

PinPoint(TM) Xa Protein Purification System

by Gregg Larson
Promega Corporation

The PinPoint(TM) Xa Protein Purification System (Cat.#V2020) is used for expression of biotin-tagged fusion proteins in E. coli. SoftLink(TM) Soft Release Avidin Resin, included with the system, is used to purify the biotinylated fusion proteins. Following are answers to some of the common questions we receive about the PinPoint(TM) System.

Q: How does the PinPoint(TM) System work?

DNA coding for the protein of interest is cloned into one of the three PinPoint(TM) Xa Vectors, which differ only in reading frame. The DNA is inserted downstream from a sequence encoding a peptide that is biotinylated *in vivo* as the fusion protein is expressed. The recombinant plasmid is transformed into *E. coli* and protein production is induced with IPTG. Cells are lysed and the biotinylated fusion protein is captured by the SoftLink(TM) Resin, a monomeric avidin resin (Cat.# V2011). After the SoftLink(TM) Resin is washed, the biotinylated fusion protein can be eluted under mild non-denaturing conditions (5mM biotin).

Q: What is the purification tag in the PinPoint(TM) System?

The purification tag is a subunit of the transcarboxylase complex from *Propionibacterium shermanii*, which is biotinylated *in vivo* by biotin holoenzyme ligase (E.C.6.3.4.9) in *E. coli* (1). This tag is also biotinylated in insect (2) and yeast (1) cells when using suitable promoters for expression. The 122 amino acid tag is expressed as an N-terminal fusion, adding about 13kDa to the molecular weight of the protein of interest. Specifically, amino acid residue 88 (lysine) of the purification tag is biotinylated.

Q: How are the biotinylated fusion proteins purified in the PinPoint(TM) System?

SoftLink(TM) Soft Release Avidin Resin is used for the purification of PinPoint(TM) Xa biotinylated fusion proteins or other biotinylated proteins under conditions that will maintain biological activity. The Resin is a rigid acrylic matrix coupled with monomeric avidin. The native tetrameric form of avidin has a strong binding affinity for biotin with a Kd of 10^-15. This strong affinity prevents release of captured biotinylated protein molecules under conditions that preserve biological activity. However, the SoftLink(TM) Monomeric Avidin Resin has a Kd of 10^-7, which allows gentle release of captured molecules. The SoftLink(TM) Resin will reversibly bind 20-40 nmoles of biotinylated protein per ml of Resin. Up to 4mg of biotinylated protein has been purified per ml of Resin.

Q: How much fusion protein will be purified in the PinPoint(TM) System?

One to five milligrams of protein are typically purified from 1 liter of culture lysate using the SoftLink(TM) Resin. In some cases, yields of greater than 10mg of protein are obtained when the fusion encodes a large polymeric fusion protein, such as beta-galactosidase (2).

Q: How is the biotinylated fusion protein detected in the PinPoint(TM) System?

The biotinylated fusion tag can be detected in crude lysates or in purified samples on a blot by using the Streptavidin Alkaline Phosphatase (Cat.# V5591), which is included in the PinPoint (TM) System. Briefly, a crude lysate or purified protein preparation is fractionated on an SDS polyacrylamide gel and transferred onto a membrane. The membrane is blocked, then probed with Streptavidin Alkaline Phosphatase. Detection is performed with Western Blue(TM) Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841) or another NBT/BCIP colorimetric substrate, or with a chemiluminescent substrate. A Western blot using an antibody directed against the target protein can also be performed to detect the target protein. The fusion protein will appear about 13kDa larger than the native protein on these blots due to the presence of the tag.

Q: Are proteins expressed in the PinPoint(TM) System biologically active?

Scientists at Promega have been able to express several soluble fusion proteins of various molecular weights that exhibited at least some biological activity (2). These proteins (and their fusion protein molecular weights) include beta-galactosidase (129kDa), beta-glucuronidase (82kDa), luciferase (74kDa), chloramphenicol acetyltransferase (39kDa), neomycin phosphotransferase (42kDa), and Kemptide (14kDa). The activity of the fusion protein obtained will be protein dependent. While the PinPoint(TM) System may not produce protein yields as high as some systems, it may provide a means to produce soluble active protein.

Q: What cells have been used for expression of PinPoint(TM) fusion proteins?

PinPoint(TM) fusion proteins produced at Promega have been expressed and biotinylated in *E. coli* cell lines JM109 and HB101. Theoretically, the biotin ligase should be conserved in all *E. coli* strains, although other strains have not been tested.

Q: Can the PinPoint(TM) purification tag be removed from the protein of interest?

The PinPoint(TM) Vectors encode a Factor Xa protease cleavage site on a linker located between the biotinylated peptide and the target protein. The Clip(TM) Fusion Protein Cleavage System (Cat # V4180) provides Factor Xa protease, a protease with a very specific recognition sequence (Ile-Glu-Gly-Arg), as well as Trypsin and Clostripain, which can also be used to cleave the purification tag at the Factor Xa site. Although, Clostripain (Arg*) and Trypsin (Lys* and Arg*) are less specific proteases than Factor Xa, they are provided as alternative enzymes because the Factor Xa cleavage site between some fusions may be resistant to cleavage with Factor Xa protease. Clostripain and Trypsin have been used to successfully cleave between PinPoint(TM) fusion partners using mild proteolytic conditions (2). The protein of interest can be purified from the fusion tag and protease by using ion exchange columns (3).

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Q: Can other proteolytic peptide sequences be inserted between the PinPoint(TM) purification tag and the protein of interest?

Amino acid sequences specific for cleavage by other proteases, e.g. enterokinase, can be engineered between the purification tag and the protein of interest at the unique *Nru I* site of the PinPoint(TM) Vectors by using linkers encoding these proteolytic sites (2).

Q: Can the PinPoint(TM) purification tag be shortened from 122 amino acids?

The DNA sequence encoding the first 47 amino acids of the PinPoint(TM) purification tag can be deleted from the PinPoint(TM) Vectors while still allowing expression of a biotinylated fusion protein. However, the efficiency of the biotinylation will be decreased (1). For best results, we recommend expressing the entire length of the 122 amino acid purification tag.

References

1. Cronan, J.E., Jr. (1990) *J. Biol. Chem.* **265**, 10327.
2. Cress, D., Shultz, J., and Breitlow, S. (1993) *Promega Notes* **42**, 2.
3. *Clip(TM) Fusion Protein Cleavage System Technical Bulletin,* #TB180, Promega Corporation.

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EXHIBIT

3

Protein Modification Reagents

Protein Modification Reagents

Proteins may be modified *in vitro* so that they may be used for detection, purification and assay development. In very general terms, protein modification reagents can be separated into reagents that add, cleave or reduce. Reagents that add labels are used for immunoassays, flow cytometry, fluorescence-activated cell sorter (FACS) analysis and molecular structure and function studies. Addition reagents also include those used to block particular functional groups. Reagents that enzymatically cleave can be used for removing amino acids, producing antibody fragments and releasing peptides from fusion proteins. Reagents that chemically reduce can be used for protein solubilization and to facilitate cross-linking.

Label Addition Reagents

Using many of the same reactive chemistries incorporated into cross-linking reagents (see previous topic in this catalog section), a variety of modification reagents have been developed for adding labels or blocking functional groups on proteins and other molecules. A number of different affinity or detectable (e.g., fluorescent) labels have been manufactured in activated form ready for covalent coupling to proteins and other molecules. For example, Sulfo-NHS-LC-Biotin (Product # 21335) is a biotin label bearing a succinimidyl ester group that enables it to be covalently attached to primary amines on proteins. Biotin, fluorescent and other non-isotopic labeling methods are discussed more fully in the Protein Labeling section.

Proteins and other molecules can be labeled with a radioactive element for use in applications that require an extremely sensitive means of detection, such as localization and imaging studies. Some of the more common radiolabels are ^{14}C, ^{32}P, ^{35}S, ^{3}H and the iodine isotopes ^{125}I and ^{131}I. Radioiodination is the process of labeling the hydroxyl group on tyrosine residues. When tyrosines are unavailable or in low abundance, a variety of reagents are available that can introduce phenolic sites (Table 6), or the imidazole ring of histidine can be labeled by iodinating at pH >9.

Table 6. Modification Reagents that can be Iodinated*

Reagent	Reactivity	Product #
Bolton-Hunter Reagent (SHPP)	Primary Amines	27710
Water-Soluble Bolton-Hunter Reagent (Sulfo-SHPP)	Primary Amines	27712
Succinimidyl-3-(tri-*N*-butylstannyl) benzoate	Primary Amines	27776
Sulfo-SHB	Primary Amines	27775
HPPH	Carbonyl/Aldehyde	27772
ß-(4-Hydroxyphenyl)ethylmaleimide	Sulfhydryl	27770
ß-(4-Hydroxyphenyl)ethyl iodoacetamide	Sulfhydryl	27771

*See Protein Labeling Section for more information.

Addition Reagents that Alter or Block Functional Groups

In many applications, especially those involving cross-linking or detection of specific functional groups, it is sometimes necessary to selectively block one functional group (e.g., amines) or else add more of one particular functional group (e.g., add more sulfhydryl groups) to one or more proteins used in an experiment. Blocking functional groups is also useful for reducing background detection in certain assays.

As with labeling reagents, most of these modification reagents use the familiar reaction chemistries already discussed for cross-linkers. For example, Sulfo-NHS-Acetate (Product # 26777) uses the succinimidyl ester chemistry to acylate and thereby block primary amines on proteins or other amine-containing molecules. Some blocking reagents are reversible, allowing protection during a particular procedure followed by restoration to the original functional group. Citraconic Anhydride (Product # 20907) is one such reagent for reversible blocking of primary amines.

Traut's Reagent and SATA (Product # 26101 and 26102) are two reagents that add sulfhydryls to primary amines. They are particularly useful when preparing enzyme-antibody conjugates using the heterobifunctional cross-linker Sulfo-SMCC (Product # 22322). SATA is unique in that it adds a sulfhydryl in a protected form, which may be deprotected when needed using Hydroxylamine•HCl (Product # 26103).

Enzymatic Cleavage Reagents

Enzymes may be used as catalysts to effect a wide variety of biochemical transformations. Whether dissolved in solution or immobilized to an insoluble support, enzymes can specifically cleave proteins at discrete sites to isolate fragments of known activity or structure. Such cleavage events also can be a way to demonstrate protein purity by analyzing what peptide fragments result from proteolysis. Pierce proteolysis products include highly purified enzymes that can be used in buffered solutions, immobilized enzymes that facilitate simple removal of protease activity after use, assays to monitor protease activity, and protease inhibitors to avoid proteolysis in biological samples.

Enzymes with specific cleavage characteristics that recognize the amino acid sequence of a protein chain, such as Factor Xa (Product # 32520, 32521), *Staphylococcus aureus* Protease (Product # 20195), Submaxillaris Protease (Product # 20199), and TPCK Trypsin (Product # 20230, 20233) may be used to effect cleavage events at particular sites within proteins. For instance, Factor Xa cleaves the last amino acid in the sequence Ile-Glu-Gly-Arg. Peptide linkers can be designed with this sequence between two recombinant fusion proteins, and once the protein has been expressed with a fusion tag to facilitate detection or isolation, the two proteins can be cleaved using Factor Xa to isolate the desired protein.

Some enzymes that are not particularly discrete with regard to their cleavage site, such as pepsin and papain, have nonetheless found important application in fragmenting antibodies. These enzymes will cleave at two different points at the hinge region of IgG molecules (the most accessible polypeptide region of immunoglobulins), forming fragments containing either one or two antigen binding regions. Antibody fragmentation using enzymes is important for removing nonspecific binding and the effector functions of the Fc region, while creating smaller molecules still able to interact with antigens. For more information on antibody fragmentation, see the Antibody Production and Purification section.

Reducing Disulfide Bonds

Reduction of disulfide bonds in proteins may be required or beneficial for a number of reasons:

- Exposure of sulfhydryls for cross-linking
- Protein activation
- Oligomer separation
- Protein denaturation
- Protein solubilization
- Disulfide bond characterization
- Protection of protein thiols from oxidation

Generally, free thiol groups are excellent targets for attaching a tag or enzyme label to a protein. Not only are sulfhydryl-reactive cross-linkers readily available, but the abundance of sulfhydryl groups in proteins is much lower than other sites used for modification, allowing more focused modifications that are less likely to inactivate or block protein function.

Reducing agents include those that contain thiol groups such as dithiothreitol (DTT), 2-mercaptoethanol and 2-mercaptoethylamine, and those that are phosphines and their derivatives, such as Tris(carboxyethyl) phosphine (TCEP).

In solution, sulfhydryl groups are susceptible to both oxidation and disulfide formation. Intracellular proteins generally lack disulfides, whereas extracellular proteins characteristically contain them.[1] The presence of disulfides in extracellular proteins provides structural strength. Within a cell the combination of a low redox environment and the presence of glutathione and other naturally occurring reducing agents helps to prevent disulfide formation and oxidation. Once a cell has been disrupted, oxidation and disulfide formation is favored unless a reducing agent and EDTA are present. EDTA chelates divalent cations that increase disulfide formation.[2] Overexpressed proteins in bacterial systems may become insoluble if disulfide formation is important for proper protein folding, thus causing malformed intra- and inter-disulfide bonds.

Selecting a reducing agent, as well as appropriate conditions for its use, depends on the specific application. To selectively reduce disulfide bonds between heavy chains of an antibody, a low concentration of 2-Mercaptoethylamine•HCl (2-MEA, Product # 20408) is used. Other reductants in limiting concentrations also can be used. The strong reducing agent 2-Mercaptoethanol (Product # 35602) is used in sample preparation for electrophoresis and isoelectric focusing, and complete reduction of disulfide containing molecules. Generally, 2-mercaptoethanol is used at a final concentration of 0.1-0.5% and pH 6-9. Disadvantages of 2-mercaptoethanol are its distinct, pungent odor and its instability.

DTT (Product # 20290, 20291) can be substituted in most applications that use 2-mercaptoethanol. The optimal pH range for DTT is between 7.1 and 8.0, but the reagent can be used effectively at pH 6.5-9.0. DTT supplied as a powder has a longer shelf life than 2-mercaptoethanol; however, stock solutions must be used immediately and any remaining solution discarded. To maintain reduced proteins in solution, DTT is used at 1-10 mM, and for complete reduction for electrophoresis, 50-100 mM is used.

TCEP (Product # 77720, 20490, 77712) can be used in many applications that call for 2-mercaptoethanol or DTT, with the exception of isoelectric focusing because TCEP is a charged molecule. TCEP has a much greater redox potential than DTT, so generally only half the concentration of TCEP is needed compared with DTT for the same application. TCEP, however, is not able to penetrate into hydrophobic cores where many disulfides may reside. Therefore, the addition of a denaturant (e.g., a detergent) or adjustment to acidic (e.g., pH 2.8-5) or basic (pH 9-11) conditions is often necessary to effect complete reduction. TCEP retains complete reducing ability across a large pH range (pH 2-11).

Once reduction is complete, removal of excess reducing reagents from the sample must be performed rapidly to prevent reformation of the disulfide and oxidation. Desalting can be performed; however, the sample becomes diluted, and small molecular weight samples, such as peptides are difficult to separate from the reducing agent. Using immobilized reducing agents, such as Reduce- Imm Reducing Kit (Product # 77700) and Immobilized TCEP Disulfide Reducing Gel (Product # 77712) eliminate these difficulties. Furthermore, soluble reducing agents may interfere with the assay used for determination of free thiol groups in the sample, or prevent coupling of the free thiol to a sulfhydryl reactive reagent or various other downstream applications. Immobilized reducing agents, therefore, offer several advantages compared to soluble reducing agents.

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References

1. Methods in Molecular Biology: Protein Stability and Folding, Theory and Practice. Vol. 40, Bret A. Shirley, ed. 1995.
2. Slopes, R.K. 1982, pp. 185-193, Protein Purification: Principles and Practice, Springer-Verlag, New York.

To return to:

- Protein Structure Main page click here.
- Protein Pathways Main page click here.

Other Sites of Interest

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EXHIBIT

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New England Biolabs, Inc.
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pMAL™ Protein Fusion and Purification System

#E8000S ..... $400 (USA)

Description

In the Protein Fusion and Purification System, the cloned gene is inserted into a pMAL vector down-stream from the *malE* gene, which encodes maltose-binding protein (MBP). This results in the expression of an MBP-fusion protein (1,2,3). The technique uses the strong P_{tac} promoter and the translation initiation signals of MBP to express large amounts of the fusion protein. The fusion protein is then purified by a one-step affinity purification for MBP (4).

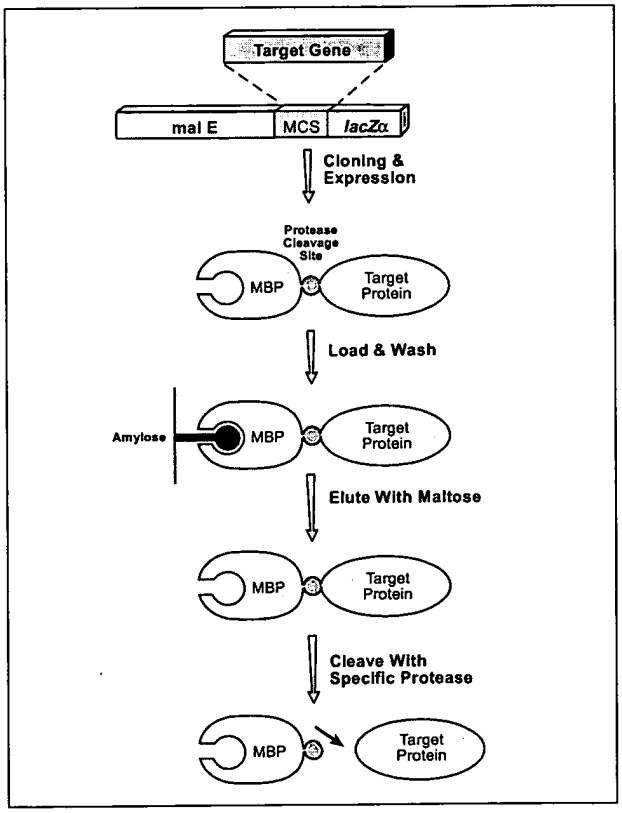
The System Includes

- pMAL-c2X: 10 µg
- pMAL-p2X: 10 µg
- Amylose Resin: 15 ml
  (binding capacity ~40 mg)
- Factor Xa: 50 µg
- anti-MBP antiserum: 25 µl
  (for Western blot analysis)
- MBP2*: 10 µg
  (marker for SDS-polyacrylamide gels)
- MBP2-paramyosin-ΔSal: 100 µg
  (control for Factor Xa cleavage)
- *E. coli* Host
- A Comprehensive Instruction Manual

Method Overview

The system uses the pMAL vectors which are designed so that insertion interrupts a *lacZ*α gene allowing a blue-to-white screen for inserts on X-gal (5). pMAL-c2 series has an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. pMAL-p2 series contains the normal *malE* signal sequence, which directs the fusion protein through the cytoplasmic membrane. pMAL-p2 fusion proteins capable of being exported can be purified from the periplasm. Between the *malE* sequence and the polylinker there is a spacer sequence coding for 10 asparagine residues. This spacer insulates MBP from the protein of interest, increasing the chances that a particular fusion will bind tightly to the amylose resin. The vectors also include a sequence coding for the recognition site of a specific protease. This allows the protein of interest to be cleaved from MBP after purification, without adding any vector-derived residues to the protein (6). For this purpose, the polylinker includes a restriction site superimposed on the sequence coding for the site of the specific protease. This is where the gene of interest is inserted. An *Eco*R I site in the same reading frame as that of λgt11 and a number of other useful sites are present directly downstream. The vectors also include the M13 origin of DNA replication which allows the production of single-stranded DNA for sequencing and mutagenesis by infecting with M13KO7 helper phage (NEB #N0315S).

*Patent #5,643,758



Expected Results

Expression from the pMAL vectors yields up to 100 mg fusion protein from a liter of culture. While no expression system works with every cloned gene, the pMAL Protein Fusion and Purification System gives substantial yields of protein in about 75% of the cases tested. A chapter in *Current Protocols in Molecular Biology* (3) provides an in-depth analysis of the use of the pMAL vectors. The System's instruction manual is available separately upon request.

References

1. Guan, C. et al. (1987) *Gene* 67, 21–30.
2. Maina, C.V. et al. (1988) *Gene* 74, 365–373.
3. Riggs, P., in Ausebel, F.M. et al. (eds), *Current Protocols in Molecular Biology* Greene Associates/Wiley Interscience, New York pp. 16.6.1–16.6.10.
4. Kellerman, O.K. and Ferenci, T. (1982) *Methods in Enzymol.* 90, 459–463.
5. Yanisch–Perron, C. et al. (1985) *Gene* 33, 103–119.
6. LaVallie, E., in Ausebel, F.M. et al. (eds), *Current Protocols in Molecular Biology* Greene Associates/Wiley Interscience, New York pp. 16.4.1–16.4.17.

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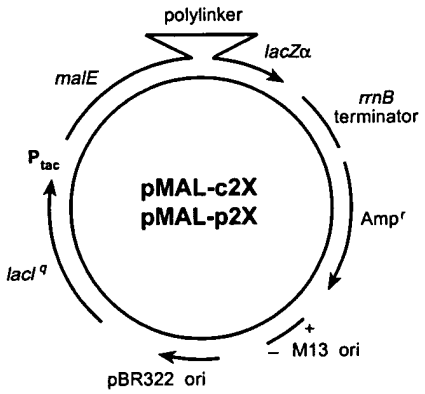
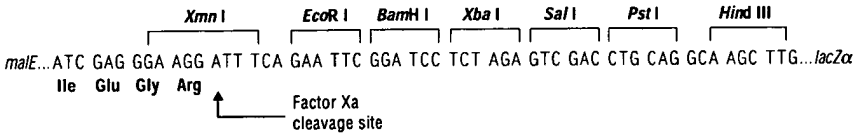
System Components & Companion Products

Vectors

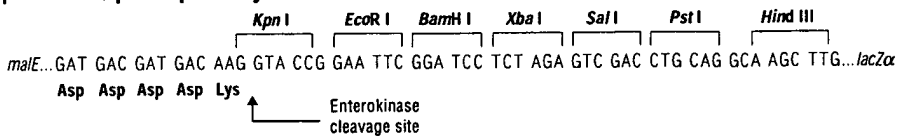
The pMAL-c2 series of vectors have an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. The pMAL-p2 series of vectors contain the normal *malE* signal sequence, which directs the fusion protein through the cytoplasmic membrane. All of the vectors include a sequence coding for the recognition site of a specific protease (Factor Xa [X], Enterokinase [E] or Genenase™ I [G]) which allows the protein of interest to be cleaved from MBP after purification.



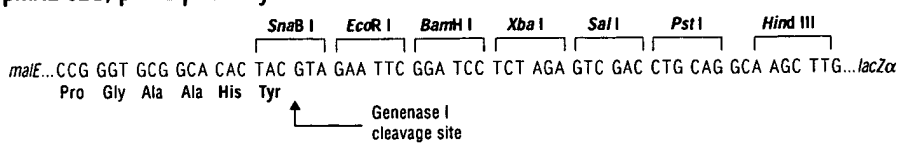
pMAL™-c2X, -p2X polylinker:



pMAL-c2E, pMAL-p2E Polylinker



pMAL-c2G, pMAL-p2G Polylinker



pMAL™ Vectors

pMAL-c2X
#N8076S 10 µg .....$75 (USA)

pMAL-p2X
#N8077S 10 µg .....$75 (USA)

Note: pMAL-c2X and pMAL-p2X are supplied with the pMAL Protein Fusion and Purification System (NEB #E8000S).

pMAL-c2E *(not supplied with the system)*
#N8066S 10 µg .....$75 (USA)

pMAL-p2E *(not supplied with the system)*
#N8067S 10 µg .....$75 (USA)

pMAL-c2G *(not supplied with the system)*
#N8068S 10 µg .....$75 (USA)

pMAL-p2G *(not supplied with the system)*
#N8069S 10 µg .....$75 (USA)

System Components & Companion Products *(continued)*

Amylose resin

#E8021S	15 ml	..... $100 (USA)
#E8021L	100 ml	..... $300 (USA)

Factor Xa

#P8010S	50 µg	.....$50 (USA)
#P8010L	250 µg	..... $200 (USA)

Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate (1,2,3). A number of the secondary sites have been sequenced that show cleavage following gly-arg. Acetylation of the γ amino group of lysine has been shown to block nonspecific cleavage at lysine residues (4). There seems to be a correlation between proteins that are unstable in *E. coli* and those that are cleaved by Factor Xa at secondary sites; this may indicate that these proteins are in a partially unfolded state (Hall, I., Riggs, P., unpublished observations).

Source: Factor Xa protease is purified from bovine plasma.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of test substrate to 95% completion in 6 hours or less.

Unit Assay Conditions: 20 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$ (pH 8.0), 50 µg of an MBP fusion protein test substrate and enzyme. Incubate at 23°C.

References:

1. Nagai, K. et al., (1985) *PNAS USA* 82, 7252–7255.
2. Quinlan, R.A. et al., (1989) *J. Cell Sci.* 93, 71–83.
3. Eaton, D. et al., (1986) *Biochem.* 25, 505–512.
4. Wearne, S.J., (1990) *FEBS Lett.* 263, 23–26.

Enterokinase *(not supplied with the system)*

#P8070S	5 units	.....$80 (USA)
#P8070L	25 units	.....$320 (USA)

Enterokinase is a specific protease that cleaves after the lysine in its preferred cleavage site Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate.

Source: Enterokinase is purified from *E. coli* containing a clone of the light chain of the bovine enterokinase gene, fused to a carrier protein (1,2). The fusion protein is cleaved and the enterokinase domain is purified away from the carrier.

Unit Definition: One unit is defined as the amount of enterokinase that will cleave 50 µg of a fusion protein that contains a fusion joint of asp-asp-asp-asp-lys to 95% completion in 8 hours at 23°C.

Unit Assay Conditions: 20 mM Tris-HCl, 50 mM NaCl, 2 mM $CaCl_2$ (pH 7.4 @ 25°C), 50 µg of an MBP fusion protein test substrate and enzyme in a 50 µl reaction. Incubate at 23°C.

References:

1. La Vallie, E.R. et al. (1993) *J. Biol. Chem.* 268, 23311–23317.
2. La Vallie, E.R. and Racie, L., unpublished observations.

Genenase™ I *(not supplied with the system)*

#P8075S	50 µg	.....$50 (USA)
#P8075L	250 µg	..... $200 (USA)

Genenase I is a variant of subtilisin BPN′ that has been engineered to have increased specificity by substituting amino acids in its active site (1,2). When designing fusion proteins for cleavage with Genenase I, we recommend the site Pro-Gly-Ala-Ala-His-Tyr. Genenase I will cleave at other histidine residues depending on the surrounding amino acids and the 3-dimensional conformation of the protein. Genenase I cleaves His-Tyr-Glu and His-Tyr-Asp slowly, but will not cleave His-Tyr-Pro or His-Tyr-Ile (2).

Source: *Bacillus amyloliquefaciens,* cloned in *Bacillus subtilis.*

Unit Definition: 0.5 µg of Genenase I will cleave 50 µg of test substrate to 95% completion in 8 hours or less at 23°C.

Unit Assay Conditions: 20 mM Tris-HCl (pH 8.0 @ 25°C), 200 mM NaCl, 50 µg of MBP fusion test substrate and enzyme. Incubate at 23°C.

References:

1. Carter, P. and Wells, J.A. (1987) *Science* 237, 394–399.
2. Carter, P. et al. (1989) *Proteins: Structure, Function, and Genetics* 6, 240–248.

Genenase™ I is a trademark of Genencor International Inc.

Anti-MBP Anti Serum

#E8030S	0.2 ml	..... $100 (USA)

Rabbit serum prepared using purified maltose-binding protein; Suggested dilution for Western blotting or ELISA 1:10,000.

Anti-MBP Antibody *(not supplied with the system)*

#E8031S	0.08 ml	..... $200 (USA)

Anti-MBP Antibody is purified from serum by Protein A and maltose-binding protein affinity chromatography.

MBP2*

#E8044S	1.0 mg	.....$50 (USA)
#E8044L	5.0 mg	..... $200 (USA)

MBP2* is wild type MBP plus the amino acids encoded by the pMAL-c2 polylinker. It is purified from cells bearing a derivative of pMAL-c2 with a linker containing a stop codon inserted in the *Xmn* I site.

MBP2*-paramyosin ΔSal

#E8051S	100 µg	.....$50 (USA)

An MBP fusion protein used as a positive control for factor Xa cleavage.

(See other side)

System Components & Companion Products *(continued)*

***E. coli* host TB1**
ara Δ(*lac proAB*) *rpsL* (ϕ80 *lacZΔM15*) *hsdR*
Available upon request at no charge with an order or for the cost of shipping if ordered separately (#E4122S).

***malE* primer** *(not supplied with the system)*
#S1237S 0.5 A_{260} units .....$95 (USA)

Used for sequencing downstream from the *malE* gene across the polylinker,

5′...dGGTCGTCAGACTGTCGATGAAGCC...3′

M13/pUC sequencing primer *(not supplied with the system)*
#S1224S 0.5 A_{260} units .....$95 (USA)

Used for sequencing upstream from the *lacZα* gene across the polylinker,

5′...dCGCCAGGGTTTTCCCAGTCACGAC...3′

M13KO7 Helper Phage *(not supplied with the system)*
#N0315S 1.8 ml .....$55 (USA)

Superinfection of cultures containing pMAL-2 clones with M13KO7 results in preferential packaging of single-stranded pMAL for mutagenesis and sequencing.

Notice to Buyer/User

The buyer/user has a non-exclusive license to use the pMAL vectors for research purposes only. A license to use the pMAL vectors for the commercial purposes is available from New England Biolabs, Inc. U.S. Patent #5,643,758.

Information presented herein is accurate and reliable to the best of our knowledge and belief, but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary.

EXHIBIT

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individual-specific, limited in their recognition spectrum and difficult to obtain against most malignancies. Antibodies, on the other hand, are readily obtainable, more easily derived, have wider spectrum and are not individual-specific. The major problem of applying specific antibodies for cancer immunotherapy lies in the inability of sufficient amounts of monoclonal antibodies (mAb) to reach large areas within solid tumors.

Technical Paper Explaining Eshhar's Invention, at 6.

The inventions of Capon and Eshhar are the chimeric DNA that encodes single-chain chimeric proteins for expression on the surface of cells of the immune system, plus expression vectors and cells transformed by the chimeric DNA. The experts for both parties explain that the invention combines selected DNA segments that are both endogenous and nonendogenous to a cell of the immune system, whereby the nonendogenous segment encodes the single-chain variable ("scFv") domain of an antibody, and the endogenous segment encodes cytoplasmic, transmembrane, and extracellular domains of a lymphocyte signaling protein. They explain that the scFv domain combines the heavy and light variable ("Fv") domains of a natural antibody, and thus has the same specificity as a natural antibody. Linking this single chain domain to a lymphocyte signaling protein creates a chimeric scFv-receptor ("scFvR") gene which, upon transfection into a cell of the immune system, combines the specificity of an antibody with the tissue penetration, cytokine production, and target-cell destruction capability of a lymphocyte.

The parties point to the therapeutic potential if tumors can be infiltrated with specifically designed immune cells of appropriate anti-tumor specificity.

The Eshhar Claims

The Board held unpatentable the following claims of Eshhar's '994 application; these were all of the '994 claims that had been designated as corresponding to the count of the interference. Eshhar's claim 1 was the designated count.

1. A chimeric gene comprising

a first gene segment encoding a single-chain Fv domain (scFv) of a specific antibody and

a second gene segment encoding partially or entirely the transmembrane and cytoplasmic, and optionally the extracellular, domains of an endogenous protein

wherein said endogenous protein is expressed on the surface of cells of the immune system and triggers activation and/or proliferation of said cells,

which chimeric gene, upon transfection to said cells of the immune system, expresses said scFv domain and said domains of said endogenous protein in one single chain on the surface of the transfected cells such that the transfected cells are triggered to activate and/or proliferate and have MHC nonrestricted antibody-type specificity when said expressed scFV domain binds to its antigen.

2. A chimeric gene according to claim 1 wherein the second gene segment further comprises partially or entirely the extracellular domain of said endogenous protein.

3. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against tumor cells.

4. A chimeric gene according to claim 1 wherein the first gene segment encodes the scFv domain of an antibody against virus infected cells.

5. A chimeric gene according to claim 4 wherein the virus is HIV.

6. A chimeric gene according to claim 1 wherein the second gene segment encodes a lymphocyte receptor chain.

7. A chimeric gene according to claim 6 wherein the second gene segment encodes a chain of the T cell receptor.

9. A chimeric gene according to claim 7 wherein the second gene segment encodes the α, β, γ, or δ chain of the antigen-specific T cell receptor.

10. A chimeric gene according to claim 1 wherein the second gene segment encodes a polypeptide of the TCR/CD3 complex.

11. A chimeric gene according to claim 10 wherein the second gene segment encodes the zeta or eta isoform chain.

12. A chimeric gene according to claim 1 wherein the second gene segment encodes a subunit of the Fc receptor or IL-2 receptor.

13. A chimeric gene according to claim 12 wherein the second gene segment encodes a common subunit of IgE and IgG binding Fc receptors.

14. A chimeric gene according to claim 13 wherein said subunit is the gamma subunit.

15. A chimeric gene according to claim 13 wherein the second gene segment encodes the CD16α chain of the FcγRIII or FcγRII.

16. A chimeric gene according to claim 12 wherein the second gene segment encodes the α or β subunit of the IL-2 receptor.

17. An expression vector comprising a chimeric gene according to claim 1.

18. A cell of the immune system endowed with antibody specificity transformed with an expression vector according to claim 17.

19. A cell of the immune system endowed with antibody specificity comprising a chimeric gene according to claim 1.

20. A cell if the immune system according to claim 19 selected from the group consisting of a natural killer cell, a lymphokine activated killer cell, a cytotoxic T cell, a helper T cell and a subtype thereof.

23. A chimeric gene according to claim 1 wherein said endogenous protein is a lymphocyte receptor chain, a polypeptide of the TCR/CD3 complex, or a subunit of the Fc or IL-2 receptor.

The Board did not discuss the claims separately, and held that the specification failed to satisfy the written description requirement as to all of these claims.

The Capon Claims

Claims 1-10, all of the claims of the '221 patent, were held unpatentable on written description grounds. Claims 1-6 are directed to the chimeric DNA, claims 7, 8, and 10 to the corresponding cell comprising the DNA, and claim 9 to the chimeric protein:

1. A chimeric DNA encoding a membrane bound protein, said chimeric DNA comprising in reading frame:

DNA encoding a signal sequence which directs said membrane bound protein to the surface membrane;

DNA encoding a non-MHC restricted extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least one ligand is a protein on the surface of a cell or a viral protein;

DNA encoding a transmembrane domain which is obtained from a protein selected from the group consisting of CD4, CD8, immunoglobulin, the CD3 zeta chain, the CD3 gamma chain, the CD3 delta chain and the CD3 epsilon chain; and

DNA encoding a cytoplasmic signal-transducing domain of a protein that activates an intracellular messenger system which is obtained from CD3 zeta,

wherein said extracellular domain and said cytoplasmic domain are not naturally joined together, and said cytoplasmic domain is not naturally joined to an extracellular ligand-binding domain, and when said chimeric DNA is expressed as a membrane bound protein in a host cell under conditions suitable for expression, said membrane bound protein initiates signaling in said host cell when said extracellular domain binds said at least one ligand.

2. The DNA of claim 1, wherein said single-chain antibody recognizes an antigen selected from the group consisting of viral antigens and tumor cell associated antigens.

3. The DNA of claim 2 wherein said single-chain antibody is specific for the HIV env glycoprotein.

4. The DNA of claim 1, wherein said transmembrane domain is naturally joined to said cytoplasmic domain.

5. An expression cassette comprising a transcriptional initiation region, the DNA of claim 1 under the transcriptional control of said transcriptional initiation region, and a transcriptional termination region.

6. A retroviral RNA or DNA construct comprising the expression cassette of claim 5.

7. A cell comprising the DNA of claim 1.

8. The cell of claim 7, wherein said cell is a human cell.

9. A chimeric protein comprising in the N-terminal to C-terminal direction:

a non-MHC restricted extracellular binding domain which is obtained from a single chain antibody that binds specifically to at least one ligand, wherein said at least